plasma membrane targeting compound may be an Ena/VASP binding molecule conjugated to a plasma membrane targeting domain. Optionally the Ena/VASP binding molecule is an EVH1 binding molecule. EVH1 binding molecules include but are not limited to FPPPP peptides (SEQ ID NO.: 3) and peptide mimetics. In other embodiments the functional Ena/VASP protein is induced by expression of exogenous Ena/VASP protein in the cell.

Please delete the paragraph beginning at Page 9, line 9 through line 14 and replace it with the following:

In some preferred embodiments the Ena/VASP inhibitor is an Ena/VASP binding molecule conjugated to an intracellular targeting domain that targets Ena/VASP protein to a surface remote from the plasma membrane. The Ena/VASP binding molecule preferably is an EVH1 binding molecule, which may optionally be a FPPPP peptide (SEQ ID NO.: 3) or a peptide mimetic. In other embodiments the Ena/VASP inhibitor is an Ena/VASP antisense molecule.

Please delete the paragraph beginning at Page 13, line 14 through line 17 and replace it with the following:

Figure 5: Constitutive targeting of Ena/VASP proteins to the plasma membrane inhibits cell motility. (*a*) Schematic diagram of membrane targeting constructs (*b*) Immunofluorescence analysis of FPPPP-CAAX (SEQ ID NO.: 7) and APPPP-CAAX (SEQ ID NO.: 8) expressing cells. (*c*) Box and whisker plots of cell speed (ANOVA p-value < 0.0001).

Please delete the paragraph beginning at Page 24, line 20 through Page 25, line 9 and replace it with the following:

The crystal structure of Ena/VASP proteins has been elucidated and extensively described in the prior art. The types of structures which bind to Ena/VASP proteins is also known. For instance it is known that the EVH1 domain of Ena VASP proteins bind to peptides having the motif FPPPP (SEQ ID NO.: 3), and that the central portion of Ena/VASP proteins bind to at least three types of proteins, G-actin binding protein profilin, SH3 domains

and WW domains. The WW domain, for instance, is a small functional domain found in a large number of proteins from a variety of species including humans, nematodes, and yeast. Its name is derived from the observation that two tryptophan residues, one in the amino terminal portion of the WW domain and one in the carboxyl terminal portion, are almost invariably conserved. WW domains are about 30 to 40 amino acids in length and thus are quite small for a functional domain. In general WW domains are flanked by stretches of amino acids rich in histidine or cysteine which may be metal-binding sites. The center of the WW domains is hydrophobic, but a high number of charged residues are also present throughout the motif, which are characteristic features of functional domains involved in protein--protein interactions (*Bork and Sudol, 1994, Trends in Biochem. Sci. 19:531-533*). Among other proteins having WW domains, the rat transcription factor FE65 possesses an amino terminal activation region that includes a WW domain (*Bork and Sudol, 1994, Trends in Biochem. Sci. 19:531-533*). Src homology 3 (SH3) domains are another class of compounds that bind to Ena/VASP proteins. SH3 domains have been described extensively in the prior art. See e.g., *Pawson, 1995 Nature 373:573-580; Cohen, 1995, Cell 80:237-248 and Koch et al., 1991, Science 252:668-674.*

Please delete the paragraph beginning at Page 69, line 17 through Page 70, line 4 and replace it with the following:

The speeds of MV^{D7}/FPPPP-mito (SEQ ID NO: 3) cells were statistically indistinguishable from those of the parental MV^{D7} line, which indicates that the phenotype induced by expression of FPPPP-mito (SEQ ID NO: 3) results from a specific perturbation of Ena/VASP proteins. FACS analysis indicated that, on an average per cell basis, the MV^{D7}/EGFP-Mena cells express a level of EGFP-Mena roughly equivalent to that of the "low" population of EGFP-Mena overexpressing Rat2 cells presented in Figure 1. Because the amount of EGFP-Mena in the "low" population is similar to the amount of endogenous Mena in Rat2 cells, the MV^{D7}/EGFP-Mena cells express Mena at a level roughly comparable to that found in Rat2 fibroblasts. The MV^{D7} and MV^{D7}/EGFP-Mena cell lines were analyzed by immunofluorescence staining with probes to vinculin and F-actin and examined to verify proper distribution of EGFP-Mena. No gross differences were observed between the two cell

lines, indicating that deficiency of all Ena/VASP proteins has no effect on the appearance of focal adhesions or the distribution of F-actin. The migration rates of the MV^{D7} and MV^{D7}/EGFP-Mena cell lines were analyzed by time-lapse videomicroscopy. The MV^{D7} cells migrated significantly faster than the MV^{D7}/EGFP-Mena cells, indicating that cell speeds are reduced by complementation of the Ena/VASP-deficient cells with Mena. The results are shown in Fig. 7 When combined with the data from Rat2 cells expressing the FPPPP-mito (SEQ ID NO: 3) construct, these results provide compelling evidence that cell motility rates are increased in the absence of Ena/VASP proteins.

Please delete the paragraph beginning at Page 70, at line 30 through Page 71, line 4 and replace it with the following:

2.8 micrometer paramagnetic beads (Dynal Biotech Inc., Lake Success, N.Y.) were coated with FPPPP peptide (SEQ ID NO: 3). The beads were incubated with VASP to produce a coating of VASP on the surface of the bead. The VASP coated beads were then incubated with pre-formed actin filaments or with actin filaments that were pre-incubated with capping protein (10 nM). Filaments that were pre-incubated with capping were not captured by VASP beads, whereas uncapped filaments were. The ability of the beads to capture actin was measured.

Please delete the paragraph beginning at Page 71, line 18 through 32 and replace it with the following:

An interesting biphasic result was observed; it was found that cell speed was increased at 25nM, but decreased at 500nM. Cells expressing Ena/VASP localized at the leading edge of the cell migrated slowly as expected. Cells exposed to high levels of cytochalasin D also migrated slowly as expected. The cells receiving a low dose of cytochalasin D, however, actually demonstrated in increase in migration rate compared to the untreated cells and the cells treated with high dosages of cytochalasin D. It is believed that at the low dosage of cytochalasin D only a fraction of the barbed ends were capped leading to a decrease in average filament length, and that these shorter filaments get incorporated into structures that more

effectively move the cells forward. Presumably the high doses of cytochalasin D blocked all or nearly all of the barbed ends leading to a block of actin polymerization and consequently poor cell movement. Because the Ena/VASP proteins help elongate filaments by protecting them from capping proteins, the low-dose cytochalasin D treatment mimics the depletion of Ena/VASP protein in either the FPPPP-mito (SEQ ID NO.: 3) expressing cells or in the MVD7 cells, which are both fast moving cells.

Please delete the paragraph beginning at Page 72, line 3 through 8 and replace it with the following:

Cells positive for Ena/VASP and cells negative for Ena/VASP were examined. The actin filaments present in the leading edge of the these two classes of cells were examined by electron microscopy. For this study the cells used were: Rat2 cells (controls), Rat2/FPPPP-mito (SEQ ID NO.: 3) (fast), Rat2/FPPPP-CAAX (SEQ ID NO.: 7), Rat2/EGFP-Mena (high) (both CAAX (SEQ ID NO.: 6) and EGFP-Mena are slow). The fast-moving cells have short, highly branched actin filaments whereas slow-moving cells have long, unbranched actin filaments.

Please delete the paragraph beginning at Page 73, line 24 through Page 74, line 2 and replace it with the following:

Figure 8 is a schematic diagram depicting the known properties of Ena/VASP proteins. The properties conserved among the known Ena/VASP proteins include an EVH1 domain which is known to bind to D/EFPPPP (SEQ ID NO.: 1). The EVH1 domain is believed to play a role in focal adhesion targeting. Another conserved domain known as the proline rich domain is also shown in Figure 8. The proline rich domain binds to profilin and SH3, WW. This domain is believed to play a role in actin dynamics. A third conserved domain is referred to as EVH2. The EVH2 domain binds to actin and is involved in the mediation of actin dynamics and oligomerization. In addition to the conserved domains, two phosphorylation sites are highly conserved within Ena/VASP proteins. These phosphorylation sites are designated in Figure 8 with a *.

Please delete the paragraph beginning at Page 75, line 4 through line 11 and replace it with the following:

The differences in Listeria speed are quite significant and quite dependent on Ena/VASP proteins. This observation is accentuated by the "high dAct A5" experiments, which involved MVD7:"high" EGFP-Mena expressing cells infected with a strain of Listeria carrying a mutant ActA allele, dAct A5, that can not bind to Ena/VASP proteins (it is missing the four FPPPP repeats (SEQ ID NO.: 3) necessary for the protein-protein interaction). In contrast, wild-type Listeria are mobile in MVD7 cells that express EGFP-Mena, mutations that affect Listeria's ability to recruit Ena/VASP proteins block Listeria movement in MVD7 cells expressing EGFP-Mena.

Please amend claim 6 to read as follows:

6. The method of claim 5, wherein the EVH1 binding molecule is a FPPPP (SEQ ID NO.: 3) peptide.

In the Drawings

A Request for Approval of Proposed Drawing Corrections is enclosed along with proposed revisions to Figure 2 in which the changes are shown in red ink.

In Figure 2, after the phrase "FFFFP-mito" please add (SEQ. ID NO.: 3)

In Figure 2, after the phrase "APPPP-mito", please add (SEQ ID NO.: 4)

REMARKS

In response to an Office Action (mailed October 2, 20002) regarding the proper use of identifiers for short peptide sequences, please amend the specification and claims as set forth above. No new matter has been added.

Respectfully submitted,
Frank B. Gertler, et al., Applicant

By: _____

Helen C. Lockhart, Ph.D., Reg. No. 39,248
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2211
Telephone: (617) 720-3500

Docket No. M00656.70064.US
Date: November 25, 2002
x